

A Large Percentage of the Spanish Population Under 30 Years of Age Is Not Protected Against Hepatitis A

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A seroepidemiological study was conducted to assess the seroprevalence of hepatitis A (HAV) antibodies in the Spanish general population in 1992–93. A total of 2744 subjects (1337 men and 1407 women) in the 5–59 years age range were stratified by gender and age (5–12, 13–19, 20–29, 30–39, 40–49, 50–59 years). The presence of total anti-HAV antibodies was investigated using a commercial enzyme immunoassay. Fifty-five percent (95% CI: 53.5–57.2%) of the subjects were positive for anti-HAV antibodies, the age-standardized anti-HAV prevalence being 65.4%. Prevalence of seropositive subjects increased with increasing age ($\chi^2 = 996.17$; $P < 0.0001$), being 11%, 25% and 54% for the 5–12, 13–19 and 20–29 age groups respectively. The results from this study showed a remarkable decline in seroprevalence rates among children, adolescents and young adults. The large number of susceptible subjects in these groups of the population has public health implications in a country with intermediate HAV prevalence. *J. Med. Virol.* 60: 363–366, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: hepatitis A; seroprevalence; epidemiology

INTRODUCTION

Although hepatitis A infection occurs throughout the world, its prevalence varies widely between different geographical areas [Feinstone and Gust, 1999]. This is also the case among developed countries in the European Union for example, where marked differences are found between the seroprevalence of antibodies against hepatitis A virus (anti-HAV) in Scandinavian and southern European countries [Feinstone and Gust, 1999]. During recent decades, however, a decrease in the prevalence of anti-HAV was described in several European Mediterranean countries [Papaevangelou, 1992]. In Spain, this was first described in 1987, when

Vargas et al. [1987] compared the anti-HAV prevalence in Catalonia (a region located in the NE of Spain) in subjects 11 to 60 year old between 1977 and 1985. This study showed a remarkable seroprevalence decline in subjects of 11–30 years of age [Vargas et al., 1987]. Since then, a number of studies have confirmed this pattern. All of these seroprevalence studies, however, were conducted in specific geographical areas [Dal-Ré et al., 1991; Salleras et al., 1992; Pérez-Trallero et al., 1994; Bolumar et al., 1995; García-Fulgueiras et al., 1997] of Spain, or in specific groups (mainly in children, adolescents and university students) or selected samples [Gil et al., 1991; Lasheras et al., 1994; Rodríguez-Iglesias et al., 1995; Suárez et al., 1996; Bayas et al., 1996; Gil et al., 1998]. The only nationwide study was carried out in 1992 but comprised only young adults (20–40 years of age) [González et al., 1994]. We describe the first study aimed at assessing the seroprevalence of anti-HAV in the Spanish general population (5–59 year old groups).

MATERIALS AND METHODS

The survey was carried out on 2774 serum samples collected during 1992 and 1993 for the DRECE (Diet and Risk of Cardiovascular Disease in Spain) study, originally designed to determine the status of the Spanish population at risk of suffering cardiovascular disease [Gómez-Gerique et al., 1994] and sponsored by the Spanish Ministry of Health. Sera were frozen and stored at -20°C until anti-HAV testing was carried out. The study protocol of the present seroepidemiological survey was approved by the Research Ethics Committee of the University Clinic Hospital, Valencia, Spain.

The DRECE study was a cross-sectional study comprising the Spanish population aged 5–59 years and

Grant sponsor: SmithKline Beecham SA, Madrid, Spain.

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Accepted 11 October 1999

distributed in eight geographical regions. Cluster sampling by gender and age was carried out. Each age stratum (5–12, 13–19, 20–29, 30–39, 40–49, and 50–59 years) comprised a number of subjects proportional to the real distribution of subjects of the Spanish population. A total number of 4787 subjects were included in the study. The field work was undertaken in 53 randomly selected primary care centres. Subject recruitment was by randomized selection from the census of potential public health users and, when this was not possible, by a randomized route system. Subjects with intercurrent diseases and those who had suffered any serious disease in the three months before enrollment were not included in the study.

Laboratory Tests

The presence of total antibodies to hepatitis A virus was investigated with a commercial enzyme immunoassay (IMx HAVAB, Abbott Laboratories, Chicago, IL) according to the manufacturer's instructions. Specimens with rates equal to or lower than the cut-off rate (0.002–1000) were considered seropositive.

Sample Size Calculation

The number of sera needed to obtain a representative sample of the Spanish population per age stratum was calculated taking into account the different prevalences of anti-HAV in each stratum published in the literature [González et al., 1994; Lasheras et al., 1994; Pérez-Trallero et al., 1994]. Because the data on younger subjects (5–12, 13–19, 20–29 and 30–39 years) were of more epidemiological interest than data on older subjects (40–49 and 50–59 years), the sample sizes were calculated to obtain prevalence rates with maximum errors of $\pm 3\%$ and $\pm 5\%$ with 95% confidence (α value 0.05), for the younger and older age strata, respectively. This meant that the sample size per stratum varied from a maximum of 735 (for 20–29 years) to a minimum of 150 (50–59 years), the total number of sera being 2720. A total number of 2774 serum samples (1337 men and 1437 women) were assayed for the present seroepidemiological study. Sera were selected at random from the total number of 4787 sera collected for the DRECE study, according to the sample size calculated for each of the six strata considered.

Statistical Analysis

Differences in antibody coverage with increasing age were evaluated using chi-square test with continuity correction and the chi-square test for linear trend. Ninety-five percent confidence intervals were calculated according to the binomial exact method. All statistical analyses were carried out using SPSS 7.5 software (SPSS Inc., Chicago, IL).

RESULTS

Of the 2774 serum samples tested, 1536 (55.4%; CI: 53.5–57.2%) were positive for HAV antibodies, being 65.4% the age-standardized anti-HAV prevalence. Table I shows the prevalence of anti-HAV antibodies

TABLE I. Prevalence of Anti-HAV Antibodies According to Age Group

Age groups (years)	Number of subjects	Anti-HAV positive		
		n	%	95% CI
5–12	497	57	11.5	8.7–14.3
13–19	496	126	25.4	21.6–29.2
20–29	738	400	54.2	50.6–57.8
30–39	596	538	90.3	87.6–92.5
40–49	288	275	95.5	92.4–97.6
50–59	159	140	88.1	83.0–93.1
Total	2774	1536	55.4	53.5–57.2

Chi-square for lineal trend: $\chi^2 = 996.17$; $P < 0.0001$.

according to age groups. Prevalence of seropositive subjects increased with increasing age, ranging from 11.5% (CI: 8.7–14.3%) in the group aged 5–12 years to 95.5% (CI: 92.4–97.6%) in the group aged 40–49 years. The chi-square for linear trend was highly significant ($\chi^2 = 996.17$; $P < 0.0001$). Seroprevalence rates did not differ by gender or by geographic area. The overall seroprevalence was 727/1337 (54.4%; CI: 51.7–57.0%) in males and 809/1437 in females (56.3%; CI: 53.7–58.9%). Table II shows age-specific seroprevalence per gender.

DISCUSSION

Spain is considered to be one of the few European Union countries with intermediate anti-HAV prevalence [Feinstone and Gust, 1999]. Since the late 1970s the prevalence of anti-HAV antibody has been studied extensively in Spain, not only in the general population in some regions but also in specific groups such as gypsies [Morales et al., 1992; Cilla et al., 1995], people living in orphanages [Morales et al., 1992], mentally retarded institutionalized subjects [Gil et al., 1999], and homosexual men [Ballesteros et al., 1996].

This nationwide study confirms the results observed in previous studies conducted in specific geographical areas, i.e., the overall prevalence of hepatitis A virus infection has decreased in the general Spanish population, that is due to a remarkable decline in the prevalence among children and young adults. It is somewhat difficult to compare the results of this study with those obtained in other seroepidemiological studies in specific geographical Spanish regions, because almost every author chose different age strata when presenting the data. In any case, comparing seroprevalence data obtained in Catalonia (an autonomous region with 6 million inhabitants, 15% of the total Spanish population) in 1977 and 1985 with the data of this study showed a consistent decline over time in the seroprevalence of anti-HAV antibody among teenagers and young adults. Thus, in 1977 and 1985 anti-HAV seroprevalence was 49% and 30% for 11–20 year old subjects and 73% and 59% in the 21–30 years age group [Vargas et al., 1987], respectively, whereas our data showed that 25% and 54% in 13–19 year old and 20–29 year old subjects were seropositive, respectively. Furthermore, recent data from Catalonia showed that between 1989 and 1996, the prevalence of anti-HAV an-

TABLE II. Prevalence of Anti-HAV Antibodies According to Gender and Age Group

Age groups (years)	Males				Females			
	Number of subjects	Anti-HAV positive			Number of subjects	Anti-HAV positive		
		n	%	95% CI		n	%	95% CI
5-12	256	30	11.7	7.8-15.7	241	27	11.2	7.2-15.2
13-19	242	61	25.2	19.7-30.7	254	65	25.6	20.2-31.0
20-29	337	178	52.8	47.5-58.1	401	222	55.4	50.4-60.2
30-39	290	258	89.0	85.4-92.6	306	280	91.5	87.8-94.4
40-49	139	136	97.8	93.8-99.6	149	139	93.3	88.0-96.7
50-59	73	64	87.7	77.9-94.2	86	76	88.4	79.7-94.3
Total	1337	727	54.4	51.7-57.0	1437	809	56.3	53.7-58.9

tibody decreased by 78% in 10-14 year old subjects (from 16.2% to 3.5%), and by 27% in the 15-24 year old group (from 43.1% to 31.4%), and by 31% in 25-34 year old subjects (from 82.1% to 56.9%) [Bruguera et al., 1999].

The remarkable shift in the epidemiological pattern of anti-HAV antibody prevalence that occurred in Spain in the last decades, has been also described in other southern European countries [Papaevangelou, 1992]. This fact, most probably due to improvement of hygiene standards and sanitation, has increased the number of the susceptible children and young adults to HAV infection. This has been reported in a study conducted in Northern Spain that showed that between late 1980s and early 1990s, of the total hepatitis A cases (IgM-positive) the percentage of patients 20-39 year old increased from 32% to 49%, whereas the percentage of patients 0-19 year old decreased from 68% to 49%, respectively [Pérez-Trallero et al., 1994]. Data from Catalonia showed that among all reported hepatitis A cases between 1990 and 1995, 45% corresponded to 20-39 year old patients, whereas 26% were 10-19 year old [Bruguera et al., 1997]; the most recent data available for a full year (1997) in this region show that 20% and 40% of all reported hepatitis A cases corresponded to patients 10-19 and 20-39 year old, respectively [Direcció General de Recursos Sanitaris, 1998]. It should be mentioned, however, that data based on reported clinical hepatitis A cases reflect only part of the picture because most HAV infections in children are asymptomatic and will not be picked up in these data of reported hepatitis A cases. It seems clear that the decline in anti-HAV antibody prevalence among young adults was responsible for the increase in the number of clinical hepatitis A cases among this group of subjects, that has serious economic implications. All these data reinforce the concept that in Spain hepatitis A, the most frequent form of acute hepatitis [Buti et al., 1998], is no longer a childhood disease.

The epidemiological patterns described have clear public health implications. In the near future, an increasing percentage of teenagers and young adults will be susceptible to hepatitis A. Therefore, a number of Spanish investigators have been suggesting that universal vaccination in childhood or adolescence be implemented to help with the control of HAV infection [Lasheras et al., 1994; Pérez-Trallero et al., 1994; Rodríguez-Iglesias et al., 1995; Bolumar et al., 1995;

Suárez et al., 1996]. Recently, the Spanish Association for the Study of the Liver [Bruguera et al., 1997] recommended universal hepatitis A vaccination of 12 year old children. This is supported by several facts [Bruguera et al., 1997]: 1) prescreening is not needed, because anti-HAV antibody prevalence is currently very low in preadolescents; 2) all Spanish autonomous regions have already in place vaccination programs against hepatitis B in 11-12 year old children; 3) a hepatitis A+B combined vaccine is available; and 4) few years after the vaccination against hepatitis A is started, the number of clinical hepatitis A cases among adolescents will be almost eliminated. Some studies have estimated that the persistence of HAV-induced antibodies after the administration of three doses of HAV vaccine may last for >20 years in children and adults [Fan et al., 1998; Van Damme et al., 1994]. On the other hand, a recent study have showed that the combined hepatitis A+B vaccine given to subjects of 6-15 years is highly immunogenic after the administration of three vaccine doses [Diaz-Mitoma et al., 1999]; the geometric mean titre achieved was almost double than that reported by Fan et al. [1998] in children aged one to seven years. The high antibody titers achieved after the combined vaccine would seem consistent with long-lasting protection, assuming that the kinetics of anti-HAV decline over time would be comparable with that observed after the administration of the monovalent hepatitis A vaccine and being similar in subjects aged 1-7 years and 6-15 years. In Catalonia, 87% of preadolescents receive a full course (3 doses) of hepatitis B vaccine (Instituto de Salud Carlos III, 1998). For all the above reasons the Catalan government has decided to start, as a pilot program in Spain, the universal vaccination against hepatitis A and B to preadolescents in 1999 [Roca, 1998]. In the next few years data on the incidence of hepatitis A among adolescents (and much later among young adults) should demonstrate whether this public health approach was correct.

ACKNOWLEDGMENTS

We thank Drs. J.A. Gomez-Gerique, J.A. Gutierrez-Fuentes, M.T. Montoya and A. Porres and all the DRECE study group for supplying the samples used in the present study and M.J. Salvador for her technical assistance.

REFERENCES

- Ballesteros J, Dal-Ré R, González A, Romero J. 1996. Are homosexual males a risk group for hepatitis A infection in intermediate endemicity areas? *Epidemiol Infect* 117:145–148.
- Bayas JM, Bruguera M, Vilella A, Carbó JM, Vidal J, Navarro G, Nebot X, Prat A, Salleras LL. 1996. Prevalencia de infección por virus de la hepatitis B y hepatitis A en estudiantes de profesiones sanitarias en Cataluña. *Med Clí (Barc)* 107:281–284.
- Bolumar F, Giner-Duran R, Hernández-Aguado I, Serra-Desfilis MA, Rebagliato M, Rodrigo M. 1995. Epidemiology of hepatitis A in Valencia, Spain: public health implications. *J Viral Hepatitis* 2: 145–149.
- Bruguera M, Buti M, Diago M, García M, Jara P, Pedreira JA, Ruiz A. 1997. Indicaciones y prescripción de la vacuna de la hepatitis A en España. Informe de la Asociación Española para el estudio del Hígado. *Gastroenterol Hepatol* 20:467–473.
- Bruguera M, Salleras LL, Plans P, Vidal J, Navas E, Dominguez A, Batalla J, Taberner J LL, Espuñes. 1999. Cambios en la seroepidemiología de la infección por el virus de la hepatitis A en Cataluña en el periodo 1989–1996. Implicaciones para una nueva estrategia vacunal. *Med Clí (Barc)* 112:406–408.
- Buti M, Vargas V, Esteban R. 1998. Vacuna antihepatitis A. In: Salleras LL, editor. *Vacunaciones preventivas. Principios y aplicaciones*. Barcelona: Masson SA. p 287–292.
- Cilla G, Pérez-Trallero E, Marimon JM, Erdozain S, Gutierrez C. 1995. Prevalence of hepatitis A antibody among disadvantaged gypsy children in northern Spain. *Epidemiol Infect* 115:157–164.
- Dal-Ré R, Aguilar L, Coronel P. 1991. Current prevalence of hepatitis B, A and C in a healthy Spanish population. A seroepidemiological study. *Infection* 19:409–413.
- Diaz-Mitoma F, Law B, Parsons J. 1999. A combined vaccine against hepatitis A and B in children and adolescents. *Pediatr Infect Dis J* 18:109–114.
- Direcció General de Recursos Sanitaris. 1998. Generalitat de Catalunya. *Bulletí Epidemiològic de Catalunya* 29:175–182.
- Fan PC, Chang MH, Lee PI, Safary A, Lee CY. 1998. Follow-up immunogenicity of an inactivated hepatitis A virus vaccine in healthy children: results after 5 years. *Vaccine* 16:232–235.
- Feinstone SM, Gust ID. 1999. Hepatitis A vaccine. In: Plotkin SA, Orenstein WA, editors. *Vaccines*. 3rd ed. Philadelphia, PA: WB Saunders Company. p 650–671.
- García-Fulgueiras A, Rodriguez T, Tormo MJ, Pérez-Flores D, Chirlaque D, Navarro C. 1997. Prevalence of hepatitis A antibodies in southeastern Spain: a population-based study. *Eur J Epidemiol* 13:481–483.
- Gil A, González A, Dal-Ré R, Aguilar L, Rey J. 1991. Sero protección frente a la hepatitis A, sarampión, rubeola y parotiditis en una población escolar urbana. *Med Clí (Barc)* 96:681–684.
- Gil A, González A, Dal-Ré R, Ortega P, Dominguez V. 1998. Prevalence of antibodies against varicella zoster, herpes simplex (types 1 and 2), hepatitis B and hepatitis A viruses among Spanish adolescents. *J Infection* 36:53–56.
- Gil A, González A, Dal-Ré R, Dominguez V, Ortega P, Barrio JL. 1999. Prevalence of hepatitis A in institutionalized mentally retarded of an intermediate endemicity area. Influence of age and length of institutionalization. *J Infection* 38:120–123.
- Gómez-Gerique JA, Gutierrez JA, Porres A, Lopez D, Montoya T. 1994. The Spanish Diet and Cardiovascular Risk Factors Study (DRECE), I: Lipid Profiles (abstract). *Athero* 109(Suppl):16.
- González A, Bruguera M, Calbo F, Monge V, Dal-Ré R, Costa J y el Grupo Español de Estudio de las Hepatitis A. 1994. Encuesta epidemiológica de prevalencia de anticuerpos antihepatitis A en la población adulta joven española. *Med Clí (Barc)* 103:445–448.
- Instituto de Salud Carlos III. Ministerio de Sanidad y Consumo. 1998. Programas de vacunación frente a la hepatitis B en adolescentes. Periodo 1996–97. *Boletín Epidemiológico Semanal* 6:209–210.
- Lasheras ML, Gil A, Santos M, Rey J. 1994. Seroepidemiología del virus de la hepatitis A en niños y adolescentes. *Atención Primaria* 13:36–38.
- Morales JL, Huber L, Gallego S, Alvarez G, Díez-Delgado J, González A, Aguilar L, Dal-Ré R. 1992. A seroepidemiologic study of hepatitis A in Spanish children. Relationship of prevalence to age and socio-environmental factors. *Infection* 20:194–196.
- Papaevangelou G. 1992. Epidemiology of hepatitis A in Mediterranean countries. *Vaccine* 10(Suppl):S63–S66.
- Pérez-Trallero E, Cilla G, Urbietta M, Doronsoro M, Otero F, Marimón JM. 1994. Falling incidence and prevalence of hepatitis A in Northern Spain. *Scand J Infect Dis* 26:133–136.
- Roca J. 1998. Vacunas antihepatitis A y B: acontecimientos recientes. *Archivos de Pediatría* 49:136–139.
- Rodríguez-Iglesias MA, Pérez-Gracia MT, García-Valdivia MS, Pérez-Ramos S. 1995. Seroprevalence of hepatitis A virus antibodies in a pediatric population of southern Spain. *Infection* 23:309.
- Salleras L, Bruguera M, Vidal J, Taberner JL, Plans P, Jimenez de Anta MT, Rodes J. 1992. Cambio del patrón epidemiológico de la hepatitis A en España. *Med Clí (Barc)* 99:87–89.
- Suárez A, Navascues CA, García R, Peredo B, Miguel D, Menéndez MT, Saro C, Román F. 1996. Prevalencia de marcadores frente a los virus A y B de la hepatitis en población de Gijón entre 6 y 25 años de edad. *Med Clí (Barc)* 106:491–494.
- Van Damme P, Thoenen S, Cramm M, De Groote K, Safary A, Meheus A. 1994. Inactivated hepatitis A vaccine: reactogenicity, immunogenicity, and long-term antibody persistence. *J Med Virol* 44:446–451.
- Vargas V, Buti M, Hernández-Sánchez JM, Jardí R, Portell A, Esteban R, Guardia J. 1987. Prevalencia de los anticuerpos contra el virus de la hepatitis A en la población general. Estudio comparativo 1977–85. *Med Clí (Barc)* 88:144–146.